

What is claimed is :

1. A signal amplification system comprising a bacterial multi-hybrid system of at least two chimeric polypeptides containing :

(a) a first chimeric polypeptide corresponding to a first fragment of an enzyme;

(b) a second chimeric polypeptide corresponding to a second fragment of an enzyme or a modulating substance capable of activating said enzyme, wherein the first fragment is fused to a molecule of interest and the second fragment or the modulating substance is fused to a target ligand and wherein the activity of the enzyme is restored by the *in vivo* interaction between the said molecule of interest and the said target ligand and wherein a signal amplification is generated.

2. The signal amplification system according to claim 1, wherein the enzyme is an enzyme selected from the group consisting of adenylate cyclase and guanylate cyclase from any origin.

3. The signal amplification system according to claim 2, wherein the enzyme is the catalytic domain of *Bordetella*

adenylate cyclase (CyaA), located within the first 400 amino acid residues of the adenylate cyclase toxin.

4. The signal amplification system according to claim 3, wherein the first and the second fragments are any combination of fragments from the same enzyme which in vitro functionally interact with the natural activator of said enzyme by restoring its activity.

5. The signal amplification system according to claim 4, wherein the first and the second fragments are selected from the group consisting of :

(a) a fragment T25 corresponding to amino acids 1 to 224 of CyaA and a fragment T18 corresponding to amino acids 225 to 399 of CyaA;

(b) a fragment corresponding to amino acids 1 to 224 of CyaA and a fragment corresponding to amino acids 224 to 384 of CyaA;

(c) a fragment corresponding to amino acids 1 to 137 of CyaA and a fragment corresponding to amino acids 138 to 400 of CyaA;

(d) a fragment corresponding to amino acids 1 to 317 of CyaA and a fragment corresponding to amino acids 318 to 400 of CyaA ;

(e) two fragments from eukaryotic adenylate cyclase in association with molecules such as G protein, forskolin.

6. The signal amplification system according to claim 4 or 5, wherein the first and the second fragments are a fragment T25 corresponding to amino acids 1 to 224 of *Bordetella pertussis* CyaA and a fragment T18 corresponding to amino acids 225 to 399 of *Bordetella pertussis* CyaA.

7. The signal amplification system according to any one of the claims 1 to 3, wherein the modulating substance is a natural activator, or a fragment thereof, of the enzyme.

8. The signal amplification system according to claim 7, wherein the natural activator is the calmodulin (CaM), or a fragment thereof, and said first fragment is mutated compared to the wild type enzyme.

9. The signal amplification system according to claim 8, wherein the first fragment is a mutated fragment of the catalytic domain of *Bordetella* adenylate cyclase (CyaA).

10. A method of selecting a molecule of interest which is capable of binding to target ligand wherein the interaction between the said molecule of interest and the said target ligand is detected with a signal amplification system according to any one of the claims 1 to 9, by means of generating a signal amplification and triggering transcriptional activation.

11. The method of selecting a molecule of interest according to claim 10, wherein the signal amplification corresponds to the production of a signaling molecule.

12. The method of selecting a molecule of interest according to claim 10, wherein the transcriptional activation leads to a reporter gene expression.

13. The method of selecting a molecule of interest according to any one of claims 10 to 12, wherein the signal amplification system comprises a bacterial multi-hybrid system of at least two distinct fragments of an enzyme, whose enzymatic activity is restored by the interaction between the said molecule of interest and the said target ligand.

14. The method of selecting a molecule of interest according to any one of claims 10 to 12, wherein the signal amplification system comprises bacterial multi-hybrid system of at least a first fragment of an enzyme and a modulating substance, whose activity is restored by the interaction between the said molecule of interest and the said target ligand.

15. The method of selecting a molecule of interest according to any one of claims 10 to 14, wherein the target ligand is selected from the group consisting of protein, peptide, polypeptide, receptor, ligand, antigen, antibody, DNA

binding protein, glycoprotein, lipoprotein and recombinant protein.

16. The method of selecting a molecule of interest according to any one of claims 10 to 15, wherein the molecule of interest is capable of interacting with the target ligand and possibly of binding to said target ligand.

17. The method of selecting a molecule of interest according to any one of claims 10 to 16, wherein the interaction between the molecule of interest and the target ligand is detected, by means of signal amplification which triggers transcriptional activation, and is quantified by measuring the synthesis of the signaling molecule or the expression of the reporter gene.

18. The method of selecting a molecule of interest according to any one of claims 11, wherein the signaling molecule corresponds to the synthesis of cAMP.

19. The method of selecting a molecule of interest according to claim 11, wherein the signaling molecule corresponds to the synthesis of cGMP.

20. The method of selecting a molecule of interest according to claim 12, wherein the reporter gene expression is selected from the group consisting of gene coding for nutritional marker such as lactose, maltose ; gene conferring

resistance to antibiotics such as ampicillin, kanamycin or tetracyclin ; gene encoding for toxin; color marker such as fluorescent marker of the type of the Green Fluorescent Protein (GFP); gene encoding for phage receptor proteins or fragment thereof such as phage  $\lambda$  receptor, *lamB* and any other gene giving a selectable phenotype.

21. The method of selecting a molecule of interest according to any one of claims 10 to 20, wherein the molecule of interest is a mutant molecule compared to the known wild type molecule and said molecule of interest is tested for its capacity of interacting with the target ligand.

22. The method of selecting a molecule of interest according to any one of claims 10 to 21, wherein the selection is performed in an *E. coli* strain, or in any bacterial strain deficient in endogenous adenylate cyclase or any other eukaryotic cell.

23. A kit for selecting molecule of interest, wherein said kit comprises :

(a) a signal amplification system according to any one of claims 1 to 9;

(b) an *E. coli* strain, or in any bacterial strain deficient in endogenous adenylate cyclase or any other eukaryotic cell and ;

(c) a medium allowing the detection of the complementation selected from the group consisting of indicator or selective medium as minimal medium supplemented with lactose or maltose as unique carbon source, medium with antibiotics, medium to visualize fluorescence, conventional medium and medium which allows the sorting by the presence of the phage receptor.

24. A kit for selecting molecule of interest, wherein said kit comprises:

(a) a signal amplification system according to any one of claims 1 to 9 wherein the molecule of interest is a mutant molecule compared to the known wild type molecule;

(b) a signal amplification system according to any one of claims 1 to 9 wherein the molecule of interest is the known wild type molecule as the control;

(c) *E. coli* strain, or in any bacterial strain deficient in endogenous adenylate cyclase or any other eukaryotic cell and;

(d) a medium allowing the detection of the complementation selected from the group consisting of indicator or selective medium as minimal medium supplemented with lactose or maltose as unique carbon source, medium with antibiotics, medium to visualize fluorescence, conventional medium and medium which

allows the sorting by the presence of the phage receptor for each signal amplification system;

(e) means for detecting whether the signal amplification system with the mutant molecule is enhanced or inhibited with respect to the signal amplification system with wild type.

25. A method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest wherein respectively the stimulating or the inhibiting activity is detected with a signal amplification system according to any one of the claims 1 to 9, by means of generating an amplification and respectively of triggering or of abolishing transcriptional activation, and wherein said signal amplification and said triggered or abolished transcriptional activation are compared with those obtained from an identical signal amplification system without any substance.

26. The method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 25, wherein the signal amplification system comprises a bacterial multi-hybrid system of at least two distinct fragments of an enzyme, whose enzymatic activity is restored by the interaction between the said molecule of interest and the said target ligand.



27. The method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 25, wherein the signal amplification system comprises a bacterial multi-hybrid system of at least a first fragment of an enzyme and a modulating substance, whose activity is restored by the interaction between the said molecule of interest and the said target ligand.

28. The method of screening for substance capable of stimulating the interaction between a target ligand and a molecule of interest according to any one of claims 25 to 27, wherein the signal amplification corresponds to the production of a signaling molecule.

29. The method of screening for substance capable of inhibiting the interaction between a target ligand and a molecule of interest according to any one of claims 25 to 27, wherein the signal amplification corresponding to the production of a signaling molecule is blocked or partially abolished.

30. The method of screening for substance capable of stimulating the interaction between a target ligand and a molecule of interest according to any one of claims 25 to 28,

wherein the transcriptional activation leads to a reporter gene expression.

31. The method of screening for substance capable of inhibiting the interaction between a target ligand and a molecule of interest according to any one of claims 25 to 27 and to claim 29, wherein the transcriptional activation leading to a reporter gene expression is blocked or partially abolished.

32. The method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to any one of claims 25 to 31, wherein the target ligand is selected from the group consisting of receptor, ligand, antigen, antibody, DNA binding protein, glycoprotein and lipoprotein.

33. The method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to any one of claims 25 to 32, wherein the substance is selected from the group consisting of protein, glycoprotein, lipoprotein, ligand and any other drug having stimulating or inhibitory affinity.

34. The method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 28 or 29,

wherein the signaling molecule corresponds to the synthesis of cAMP.

35. The method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 28 or 29, wherein the signaling molecule corresponds to the synthesis of cGMP.

36. The method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to claim 30 or 31, wherein the reporter gene expression is selected from the group consisting of gene coding for nutritional marker such as lactose, maltose; gene conferring resistance to antibiotics such as ampicillin, kanamycin or tetracyclin; gene encoding for toxin; color marker such as fluorescent marker of the type of the Green Fluorescent Protein (GFP); gene encoding for phage receptor proteins or fragment thereof such as phage  $\lambda$  receptor, *lamB* and any other gene giving a selectable phenotype.

37. The method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to any one of claims 25 to 36, wherein the molecule of interest is a mutant molecule compared to the known wild type molecule and said

molecule of interest is tested for its capacity of interacting with the target ligand.

38. The method of screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest according to any one of claims 25 to 37, wherein the screening is performed in an *E. coli* strain, or in any bacterial strain deficient in endogenous adenylate cyclase or any other eukaryotic cell.

39. A kit for screening for substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest, wherein said kit comprises:

(a) a signal amplification system according to any one of claims 1 to 9 with the substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest;

(b) a signal amplification system according to any one of claims 1 to 9 without any substance as the control;

(c) *E. coli* strain, or in any bacterial strain deficient in endogenous adenylate cyclase or any other eukaryotic cell and;

(d) a medium allowing the detection of the complementation selected from the group consisting of indicator plate or selective medium as minimal medium supplemented with lactose or maltose as unique carbon source, medium with antibiotics,

medium to visualize fluorescence, conventional medium and medium which allows the sorting by the presence of the phage receptor and;

(e) means for detecting whether the signal amplification system with the substance is enhanced or inhibited with respect to the signal amplification system without any substance.

40. A molecule of interest identified by the method of any one of the claims 10 to 22.

41. A molecule of interest corresponding to a polynucleotide capable of expressing a molecule which interacts with a fused target ligand coupled with an enzyme or a fragment thereof.

42. A substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest identified by the method of any one of the claims 25 to 38.

43. The signal amplification system according to any one of the claims 1 to 9, wherein the bacterial multi-hybrid system contains:

(a) a first chimeric polypeptide corresponding to a first fragment a of an enzyme;

(b) a second chimeric polypeptide corresponding to a second fragment of an enzyme or a modulating substance capable of activating said enzyme and;

(c) a substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest, wherein the first fragment is fused to a molecule of interest and the second fragment or the modulating substance is fused to a target ligand and wherein the activity of the enzyme is restored by the interaction between the said molecule of interest and the said target ligand and wherein a signal amplification is generated.

44. Polynucleotide sequence coding for the signal amplification system according to any one of the claims 1 to 9, wherein the polynucleotide sequence codes for a bacterial multi-hybrid system of at least two chimeric polypeptides containing:

(a) a first chimeric polypeptide corresponding to a first fragment a of an enzyme fused to a molecule of interest;

(b) a second chimeric polypeptide corresponding to a second fragment of an enzyme or a modulating substance capable of activating said enzyme fused to a target ligand.

45. Polynucleotide sequence coding for the signal amplification system according to any one of the claims 1 to 9 and to claim 43, wherein the polynucleotide sequence codes for a bacterial multi-hybrid system containing:

(a) a first chimeric polypeptide corresponding to a first fragment a of an enzyme fused to a molecule of interest;

(b) a second chimeric polypeptide corresponding to a second fragment of an enzyme or a modulating substance capable of activating said enzyme fused to a target ligand;

(c) a substance capable of stimulating or inhibiting the interaction between a target ligand and a molecule of interest.